THE UNIVERSITY OF ALBERTA

INHERITANCE OF SOME CHARACTERS IN INTER- AND INTRASPECIFIC CROSSES OF MEDICAGO

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Inheritance of Some Characters in Inter- and Intraspecific Crosses of *Medicago*" submitted by Ahmet Eraç in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Inheritance studies were carried out in some intra- and inter-specific crosses in genus Medicago. The object was to find out the number of factors determining some characters and the manner of their segregation. From the results obtained it was attempted to arrive at conclusions on the relationship between taxa involved.

1. M. striata x M. littoralis. The F_1 progenies of this and the reciprocal cross showed high gametic (50%) and zygotic (26-60%) mortality, and cytoplasm-determined chlorophyll deficiencies. Segregation in most cases was not in agreement with expected normal segregation ratios. It was concluded that M. striata was not as closely related to M. littoralis as to be placed in the same species.

2. Intra-specific cross in M. intertexta. The red leaf marking was found to be a dominant character determined by one pair of alleles (M-m).

3. M. ciliaris x M. intertexta. The inheritance of red leaf marking and of hairiness indicated that these characters were determined by one pair of alleles each (M-m and H-h). The normal segregation ratios indicated that the two taxa may be considered as belonging to the same species.

4. Intra-specific crosses in M. rigidula. Inheritance of red leaf marking, of spininess and of pollen shape indicated that the inter-crossed M. rigidula strains may belong to the same species. Differential viability of gametes and zygotes may have had a disturbing effect on the segregation ratios observed. It may be assumed that wide spatial isolation of different strains have furthered accumulation of small chromosomal abnormalities causing poor pollen viability in F_1.

ACKNOWLEDGEMENTS

I wish to express thanks and appreciation to Dr. K. Lesins for his assistance and direction during the course of the investigation and preparation of this thesis; to Mrs. I. Lesins for drawing attention to certain characters in some Medicago material and for information on the growing sites of some taxa used in this study; to Dr. R. Aksel for his criticism and suggestions regarding calculations.

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INTRODUCTION AND GENERAL REMARKS

Medicago is a well-known genus because cultivated alfalfa *Medicago sativa* belongs to it. Attempts to improve the cultivated crop by hybridization has drawn attention of investigators to other species of the genus. Recently annual species have become important in their own rights as protein-rich components in pastures under dry climatic conditions (Andrew and Hely, 1960; Amor, 1965).

Often there are indistinct boundaries between different annual taxa and little work has been done for clarification of these questions. The present genetical study was undertaken to obtain insight in the inheritance of some characters possessed by some annual taxa. The data on the manner of inheritance may contribute to the evaluation of the relationships between these taxa.

The world collection of *Medicago* species and strains accumulated at the Department of Genetics, University of Alberta, Edmonton, was used as the source of material for this study. The numbers of accessions given in this study refer to numbers in that collection.

For the sake of brevity the descriptive name of the taxa in italics (underlined) have often been used instead of giving the full name, that is, omitting the name *Medicago*.

At describing crosses the maternal parent is written first unless indicated otherwise in the text.

M. striata Bast. x *M. littoralis* Rohde

The taxonomic position of *M. striata* is not well established. Bastard (1814) described it as a separate species, later Urban (1873) put it under *M. littoralis* and recently Heyn (1963) considered the taxon as

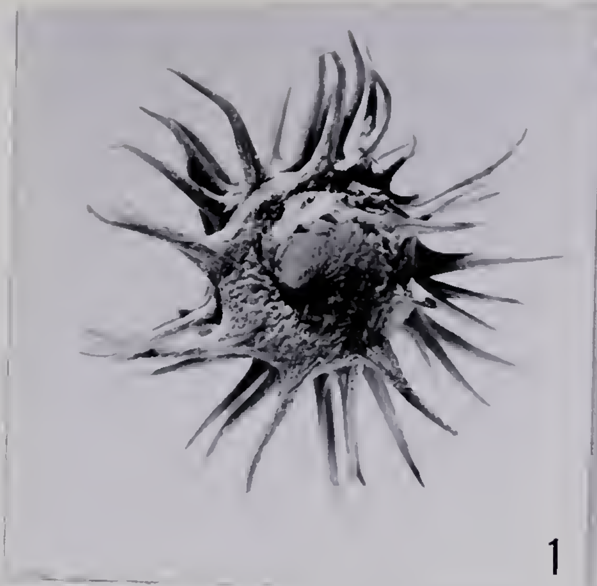
belonging to M. tornata Willd. The present study was undertaken to examine the relationship of M. striata to M. littoralis.

MATERIALS AND METHODS

M. striata was represented by the Acc. No. 1609. The material has been collected at Biarriz, France and has been described by Lesins and Lesins (1965). A characteristic pod of this strain, having very short, almost rudimentary spines, and direction of pod coiling clockwise is shown in Fig. 3. M. littoralis was of two kinds. Acc. No. 1181 was from Crete, it had pods with strong spines as shown in Fig. 1; Acc. No. 1972 had been collected in Sicily, its pods were without spines or had only short knobs instead of spines Fig. 2. Both littoralis strains had the pod coiling direction anticlockwise if looked at the pod its apex facing the observer. The two characters, spininess and anticlockwise coiling direction were used in inheritance studies. Reports on the recessiveness of the clockwise coiling direction and spinelessness in related Medicago species are found in papers by Lilienfeld and Kihara (1956), and by Simon (1965). Also in the present material the spininess was found to be dominant over the spinelessness and anticlockwise pod coiling over clockwise. In the reference papers mentioned above reverse naming for coiling direction has been used because of viewing from the base and not from the apex of the pod. M. striata as well as M. littoralis are diploid with 2n = 16 (Lesins and Lesins, 1965; Fryer 1930).

The crossing was carried out by taking buds at the stage of close-to-opening (Fig. 10A). With fine scissors a cut was made through the front of the standard in the region of calyx teeth and the upper part of the standard removed using fine forceps (Fig. 10B). After that the staminal

Figs. 1-9 Pods of *M. littoralis* 1181 (Fig. 1), *M. littoralis* 1972 (Fig. 2), of *M. striata* 1609 (Fig. 3), F_1 of *striata* x *littoralis* 1181 (Fig. 4), of *littoralis* 1972 x *striata* (Fig. 5), F_2 of *striata* x *littoralis* 1181 with spines and anticlockwise (Fig. 6), and clockwise (Fig. 7) coiling; F_2 of *striata* x *littoralis* 1972 without spines and with clockwise (Fig. 8) and anticlockwise coiling (Fig. 9).



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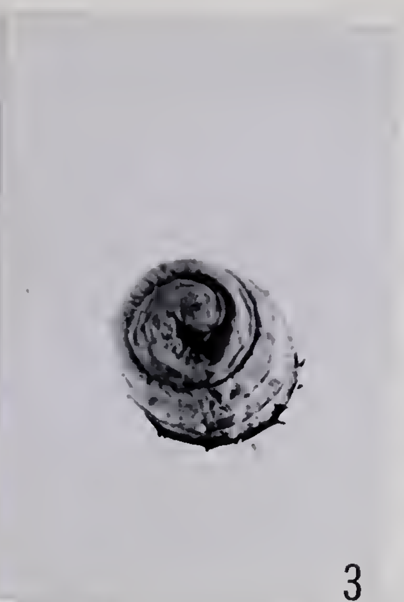

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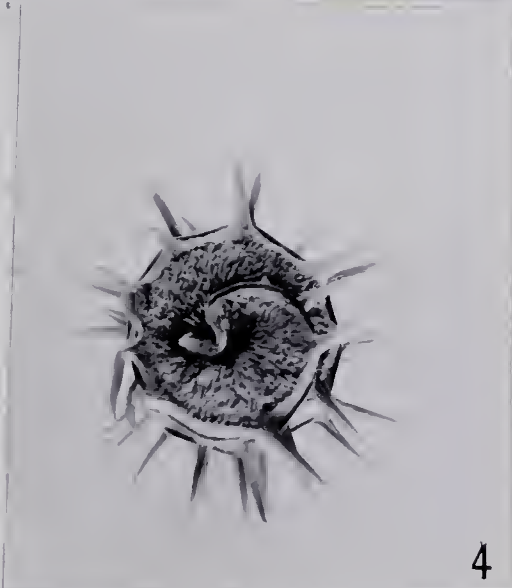

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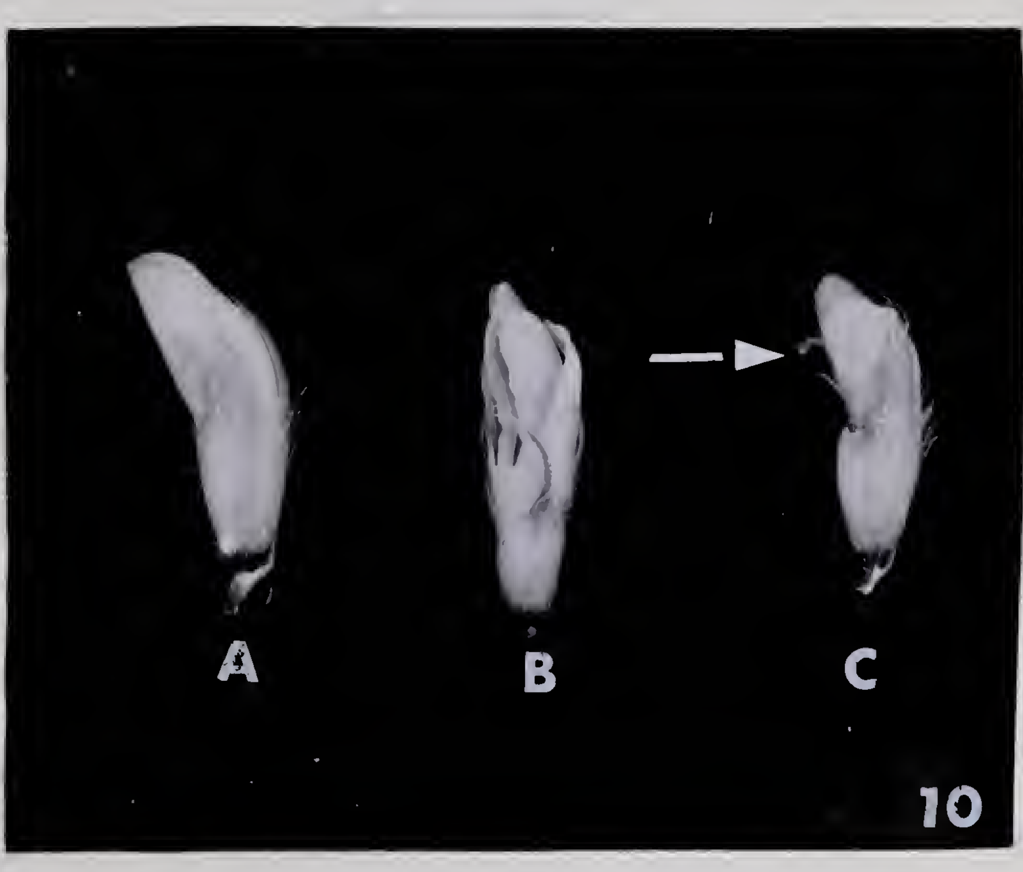
column was released by slightly spreading the keel; then the anthers and eventually released pollen grains were sucked off with a fine nozzle connect to a vacuum pump. Pollen from the paternal plant was applied with a toothpick on the exposed stigma (Fig. 10C). For identification of F_1 hybrids in crosses <u>striata</u> x <u>littoralis</u> the dominant characters of spininess and/or anticlockwise pod coiling could be used and in the reciprocal crosses an unexpected character of chlorophyll deficiency was helpful. Reliance on the above marker characters for identification of F_1 hybrids rendered the crossing much more efficient than using complete emasculation by alcohol (Lesins, 1955) or emasculation of very young buds (Simon, 1965).

Pollen viability in hybrids as compared with the parental taxa may be an indicator to the relationship of the crossing partners. Determination of pollen viability was carried out by counting plasma-filled and empty pollen grains in two 100-grain samples. The pollen from a floret was released on a slide, a small drop of liquid parafin oil was put on and cover glass applied. The counting was done from one edge of the coverglass to the opposite one in order to obtain representative counts because the empty grains tended to float more to the edges. The examination was carried out under microscope at 150X magnification, the upper lens of the condenser turned out. The plasma-containing grains had granular appearance, whereas plasma-empty grains were clear. In preliminary trials it was found that plasma-filling of the pollen grains could be determined in this manner as accurately as with the methods involving plasma staining. It should be noted that the plasma-filling determinations, with or without staining, gives only comparative values regarding the usefulness of pollen for fertilization.

Some meiotic investigations were attempted using the method described by Lesins (1954, 1963). The chromosomes of the <u>Medicago</u>, however,

Fig. 10 A - flower bud before treatment, B - upper part of the standard removed, C - stigma (arrow) exposed for crossing.


A
B
C
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are very small, 2 to 4μ in length, and in addition at the meiotic stages they tend to stick together, so only a few clearly analyzable meiotic plates were obtained: these were not considered as representative for the chromosome configurations but were useful for checking the chromosome number.

RESULTS

F_1 Generation

The *M. littoralis* No. 1972 in regard to pod morphology appeared to be closely related to *striata* (Figs. 2, 3). It was assumed that differences in other characters would be small too and the characters would follow normal segregation ratios if the two taxa belonged to one species. More attention therefore, was attached to this cross and the F_1's were produced of it in both directions. The other *littoralis* strain was used in production of F_1 only in one, that is, *striata* ♀ x *littoralis* ♂, direction.

The F_1 hybrids where *littoralis* were the pollen parents could be easily identified as soon as the pods were set (Fig. 4). Some difficulties were expected in identification of the reciprocal F_1 . However, the first 5 plants derived from the *littoralis* 1972 ♀ x *striata* ♂ cross were remarkably different from the parents and other F_1 in that they were yellowish, chlorophyll deficient; they also had smaller leaves, thinner stems and developed at a much slower rate, never reaching the vigor of normal plants. Their hybrid nature was further evidenced by pollen viability data and segregation in F_2. Repeating the cross where some selfed *littoralis* progeny were produced along with the hybrids these latter could be easily recognized by their chlorophyll deficiency as shown in Fig. 11 and verified by pollen viability. In Table I are given data on vigor and pollen viability of

parental and of F_1 plants. Vigor as expressed by stem length was measured on plants of the same age, grown under the same environmental (greenhouse) conditions.

TABLE I

Stem length cm. and percent of good (plasma-containing) pollen grains in parental and F_1 plants.

Plant material	Length of stems (cm)		% of good pollen	
	Mean	Range	Mean	Range
M. littoralis No. 1972, 10 plants	104	70-121	100	99.5-100
M. littoralis No. 1181, 10 plants	65	58- 80	87	84 - 89
M. striata No. 1609, 10 plants	71	57- 80	99	99 - 99.5
F_1 *littoralis* 1972 x *striata*, 5 plants	46	40- 53	35	32 - 38
F_1 *striata* x *littoralis* 1972, 2 plants	65	60- 69	38	32 - 43
F_1 *striata* x *littoralis* 1181, 3 plants	71	70- 72	46	41 - 50
F_1 *littoralis* 1972 x *littoralis* 1181, 38 plants			78	68 - 88

As will be seen in Table I, the vigor as expressed by stem length of F_1 *littoralis* 1972 x *striata* is quite different from that observed in parental and reciprocal F_1 plants. The percent of good pollen in all the F_1 hybrids reached scarcely one half of that in parental material. For comparison percent of good pollen from F_1 plants of the two *littoralis* strains, 1972 x 1181, where hybrids could be identified by their spininess, are given at the bottom of Table I. It will be seen that they constitute a separate group distinctly higher in good pollen than the F_1 from *littoralis* x *striata*, and reciprocal though lower than in the parental strains.

A remarkable feature, chimeras expressed as chlorophyll deficiencies in parts of leaves, was observed in some F_1 plants from *striata* x *littoralis* 1972 as well as *striata* x *littoralis* 1181 origin. From ten hybrids six had leaves with this character. It appeared sometimes in the first, unifoliate, leaf or later in trifoliate leaves (Fig. 12) involving differently large areas but of clear demarcation between the affected and normal areas. The chimeral

Fig. 11 A box with hybrid seedlings (light colored), and seedlings of selfed origin (green colored).

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portions usually appeared on different leaves again and again throughout the plant's life. A causal connection between chlorophyll deficiency comprising whole plants of *littoralis* x *striata* cross and the chlorophyll deficient plant parts in the reciprocal crosses may be suspected, because the leaflets affected showed inferior growth (Fig. 12A, B) similarly as did the whole plants in *littoralis* x *striata* cross. The general growth inhibition of the F_1 chimeral plants as compared with non-chimeral sister plants was not perceptible. F_1 chimeral plants were not found in the cross involving the two *littoralis* strains. It was obvious that chlorophyll deficiency was characteristic of the hybrid condition.

F_2 Generation

The chlorophyll deficiency phenomenon differently expressed in F_1 from reciprocal crosses was followed up in F_2 generations as shown in Table II.

The chlorophyll deficient plants from *littoralis* 1972 x *striata* were variable: some remained continuously yellowish and had only 3 to 32 cm. long stems, others gained green leaf color on maturation and attained about the same stem length, up to 90 cm., as their green sister plants though the development was slower. In the reciprocal crosses only the plants yellowish throughout showed some retarded growth while those turning green on aging attained stem length fully comparable to that of their normal sister plants, and developed at about the same rate.

As seen in Table II the conspicuous feature in the F_2 is the segregation into chlorophyll deficients and normals, in *littoralis* x *striata* the ratio mean was 6.6:1, and in the reciprocals 1:4.6 and 1:4.8, on the average 1:4.7. If the cytoplasm had been derived from the maternal side exclusively then there would not be any segregation, but if no cytoplasmic effect had occurred the reciprocals should have been identical.

Since both *littoralis* and *striata* parents had green plastids there is no question of transmission of deficient plastids to F_1 but rather the

Fig. 12 Chimeral chlorophyll deficiencies in leaves of F_1 *striata* x *littoralis*. A and B deficiencies involving whole leaflets.

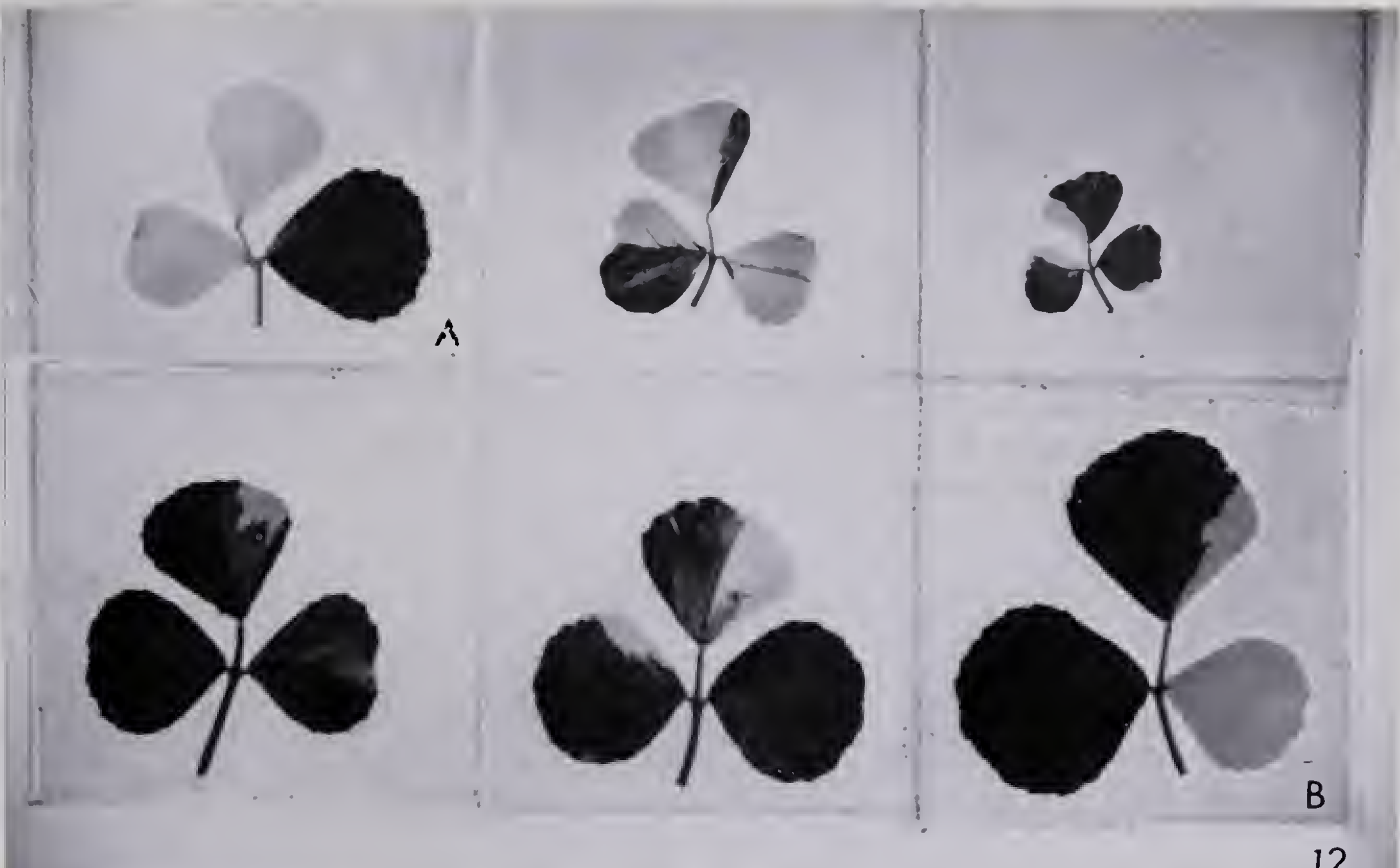

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TABLE II

Segregation of chlorophyll deficiency in F_2 from *littoralis* x *striata* and the reciprocal crosses.

Item No.	F_1 plant, origin and No.	Number of F2 Plants				
		Chlorophyll deficient				
		Seedlings die	Yellowish continuously	Green leaves on aging	Total	Normal
	littoralis 1972 x *striata*					
1	1	2	11	16	29	7
2	2	8	3	14	25	3
3	3	4	5	9	18	2
4	4	5	10	17	32	9
5	5	4	14	15	33	4
	Total	23	43	71	137	25
	striata x *littoralis* 1972					
6	7 (chimeral plant)	1	1	8 (1 chim)	10	21 (1 chim)
7	23 (not chimeral)	0	0	4	4	29
	Total	1	1	12	14	50
	striata x *littoralis* 1181					
8	2 (chimeral plant)	0	0	13	13	72
9	6 (chimeral)	1	4	12 (1 chim)	17	70 (1 chim)
	Total	1	4	25	30	142
Total *striata* x *littoralis* 1972+1181		2	5	37	44	192

detrimental influence especially of littoralis 1972 cytoplasm on the plastid development in the hybrid. The causal agent may be visualized as independently propagating plasmatic organelles with their products inhibiting normal plastid development. Organelles may be transmitted to a smaller extent by the pollen. Lesins (1961) has expressed the view that some substance in the cytoplasm detrimental for plastid development may influence the genic set-up of the hybrid so as to insure its own perpetual production.

Chimeras in the F_1 of the reciprocal cross may be visualized assuming that: (1) some cytoplasmic organelles from littoralis 1972 in small amounts may be transmitted with pollen, (2) sorting out of kinds or organelles at cell division may occur at random, (3) there is a threshold value, probably determined by the number of organelles per cell, at which the products of their activity detrimentally influence the plastid development.

These assumptions may explain the observations reported by Lesins (1961) at which deficient chlorophyll plant parts regained their green color as chimeras. Lesins (1961) explained the chimeras by influences of inner and outer environmental conditions on the labile genic set-up of the hybrids so that physiological processes could be easily channelled for production of one substance or a related one but with a different effect on the plastid development.

Segregation in F_2 is tempting in itself for assumption of gene segregation as its causal agent. In the present instance the crosses striata ♀ x littoralis ♂ may be considered as almost freed from the detrimental influence of the littoralis cytoplasm except for some chimeral patches in leaves. Under such an assumption a segregation in striata x littoralis

crosses as 3 normals to 1 chlorophyll deficient may be assumed as caused by one gene, its recessive allele giving chlorophyll deficiency. P values only in one case i.e. striata x littoralis 1181 plant No. 2 (Table II, item 8) falls outside the 5% chance variation. Moreover, some lack of chlorophyll deficient plants in this case may be explained assuming higher mortality in the chlorophyll deficient kind. Germination and survival of F_2 plants were afflicted with great mortality as seen in Table IV.

Lilienfeld (1962) assumed the segregation of two complementary genes as the basis for observed chlorophyll deficiencies in his Medicago truncatula crosses. There is no sufficient explanation for the observation that some F_2 plants remained yellowish throughout their life and some others attained green color in old leaves, but young shoots had much lighter color. This phenomenon has been reported also by Lesins (1961) and Lilienfeld (1962, 1965).

Anticlockwise coiling was dominant over clockwise direction and spininess over spinelessness. Pods from F_1 and F_2 plants are shown in Figs. 4-9 and segregation ratios are given in Table III.

As will be seen in Table III the pod coiling direction in the littoralis 1972 x striata and the reciprocal cross may fit the segregation ratio 15 anticlockwise: 1 clockwise taken separately as well as together. Lilienfeld and Kihara (1956) reported 3:1 ratio in M. littoralis strains. The striata x littoralis 1181 plant No. 2 gave 3:1 ratio whereas No. 6 gave a fit to 15:1 and the total, of course, did not fit either ratio. Spininess segregated in both plants of striata x littoralis 1181 as 3:1 with a good fit. It was tempting to calculate whether linkage was involved in factors determining coiling direction and spininess in the plant No. 2 of striata x littoralis 1181 (Table III, item 8) where a good fit to 3:1 ratio of both

characters was found. Expected for 9:3:3:1 ratio were 45:15:15:5 and found 49:13:13:5 with a very good fit of P=0.8-0.9 (P values calculated according to Snedecor, 1956).

The observed ratios may be explained considering the rate of gametic mortality as shown as percent of good pollen in Table I and high zygotic mortality as indicated, by survival of seeds and seedlings from F_1 plants as given in Table IV.

As may be seen from data in Table IV the survival of seeds from F_1 plants has ranged from 84 to as low as 40 percent in contrast to 100% survival in parental strains. If some linkage happened to tie an allele responsible for expression of a character with factors responsible for gametic or seed mortality then there will be deficiency in ratios expression: non-expression of this character.

CONCLUSIONS

It is difficult to arrive at a conclusion regarding the closeness of relationship between M. striata and M. littoralis. The morphologically closest to striata was littoralis 1972. However, they were separated by a reproduction barrier as expressed in (1) poor gametic survival, less than 50% of pollen found good in F_1 plants, (2) cytoplasmically determined chlorophyll deficiency if littoralis 1972 was the maternal side. The chlorophyll deficiency was detrimental to development of F_1 plants and was strongly felt also in F_2 generation (3) high mortality of seeds from F_1 plants (4) segregation ratios of pod coiling direction probably distorted. For pure littoralis strains Lilienfeld and Kihara (1956) has shown a segregation ratio as being 3:1. These genetically based reproduction and gene flow inhibitions would place these taxa in the rank of separate species.

TABLE III

Segregation of coiling direction and spininess in F_2 of *littoralis* x *striata* and the reciprocal crosses

Item No.	F_1 plant, origin, and No.	Number of F_2 plants					
		Coiling direction		P Value	Spininess		P Value
		Anti-clockwise	Clock-wise		With spines	Without spines	
	Littoralis 1972 x *striata*						
1	1	14	0				
2	2	16	0				
3	3	6	1				
4	4	15	0				
5	5	16	1				
	Total	67	2	0.20-0.30 (15:1)			
	striata x *littoralis* 1972						
6	7	21	3				
7	23	28	2				
	Total	49	5	0.30-0.50 (15:1)			
	striata x *littoralis* 1181						
8	2	62	18	0.50-0.70 (3:1)	62	18	0.50-0.70 (3:1)
9	6	76	8	0.20-0.30 (15:1)	59	25	0.30-0.50 (3:1)
	Total	138	26	$P < 0.01$ (3:1) $P < 0.01$ (15:1)	121	43	0.70-0.80 (3:1)

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TABLE IV

Survival of plants from seed of parental *M. striata* and *M. littoralis* strains and from their F_1 hybrids

Origin of seed	Number of seeds		Plants survived		
	Out for germination	Germinated	Number	% from germinated seed	% from total seed
M. striata	18	18	18	100	100
M. littoralis 1972	17	17	17	100	100
M. littoralis 1181	18	18	18	100	100
Total parental strains	53	53	53	100	100
F_1 *littoralis* 1972 x *striata*					
pl. No. 1	50	44	36	82	72
pl. No. 2	43	36	28	78	65
pl. No. 3	50	27	20	74	40
pl. No. 4	50	48	42	88	84
pl. No. 5	50	46	37	80	74
Total	243	201	163	81	67
F_1 *striata* x *littoralis* 1972					
pl. No. 7	75	52	31	60	41
pl. No. 23	75	52	33	63	44
Total	150	104	64	62	43
F_1 *striata* x *littoralis* 1181					
pl. No. 2	140	120	85	71	61
pl. No. 6	140	124	87	70	62
Total	280	244	172	70	61

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On the other hand the spiny strain of littoralis 1181 at least in one plant gave almost a perfect fit to 3:1 ratio for segregation both of coiling direction and spininess which would indicate that chromosomal pairing and segregation may be normal as encountered in crosses involving partners of the same species. Some pollen inferiority was observed in F_1 plants between the two littoralis strains, this would indicate that there may be gradations in inhibition of gene flow within the M. littoralis species itself; M. striata no doubt, is less closely related to these littoralis strains than they are to each other. It may be noted that Simon (1965) reported a very low percent, only 6.75% of good pollen in F_1 between M. littoralis and M. tornata. This would indicate that M. striata is closer to M. littoralis than to M. tornata. This is contrary to the views expressed by Heyn (1963).

Inheritance of Leaf Marking in M. intertexta Mill.

The triangular red marking at the base of the leaflets (Fig. 13C) of some intertexta strains sometimes has been used to separate the strain carrying it as a variety variegata Urb. Since there were no reports on the inheritance of this character the present study was carried out to investigate it.

MATERIALS AND METHODS

The red leaf marking was present in the Accession No. 239 originally obtained from Botanical Garden at Birmingham. Several tens of seeds from the original stock have been grown with the result that no segregation of the character had been observed. The nonmarked strain was Acc. No. 760b (Fig. 13A), originally collected in Sicily. It has not been observed to

Fig. 13 Leaf marking in _M. intertexta_. A - Acc. No. 760b (non-marked), B - F_1 (marked), C - Acc. No. 239 (marked).


A
B
C
13

segregate either. It was suspected that red-marking may be dominant, therefore crosses were started using red-marked No. 239 as the paternal parent. The technique of crossing was the same as described in the section dealing with M. striata x M. littoralis crosses. Some of the progeny from the non-marked ♀ x red-marked ♂ indeed showed the red leaf-marking so those undoubtedly were F_1 hybrids (Fig. 13B) and could be used for producing F_2 families. The non-marked plants were selfs as the technique of crossing allowed some self-pollination to take place. M. intertexta is known to be diploid with 2n = 16 (Fryer, 1930).

The segregation data of the red leaf marking character are given in Table V.

TABLE V

Segregation of red leaf marking in F_2 generation of the M. intertexta No. 760b x M. intertexta No. 239

Designation of F_1 plants	Number of F_2 plants		P value for 3:1 ratio
	With leaf marking	Without leaf marking	
No. 1	18	4	0.30 - 0.50
No. 2	23	9	0.50 - 0.70
No. 3	31	8	0.50 - 0.70
No. 4	22	6	0.50 - 0.70
Total	94	27	0.30 - 0.50

As may be seen in Table V the leaf marking has shown a segregation of 3 marked to 1 non-marked plant and hence it can be concluded that 1 pair of alleles is involved and that the allele "M" ("M" for marked) is dominant over that of "m" ("m" for non-marked). The relationship of the strains with and without leaf marking should be considered as close as expected for crosses within the same species.

M. ciliaris Willd x M. intertexta Gaertn

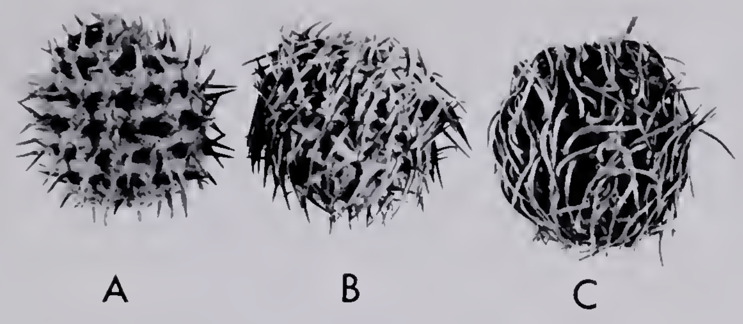
Urban (1873) considered the above two taxa as distinct species, Heyn (1963) treated them as varieties, M. intertexta var. ciliaris Heyn and M. intertexta Mill var. intertexta, respectively. The distinguishing characteristics of the two taxa are as follows: In ciliaris there are many celled glandular hairs on the pod surface (Fig. 15) especially on the dorsal nerve and on spines; the pods are 10-12 mm. in diameter; the spines are inserted at a wide angle, to the surface of the side of the coil so that the boundaries of the individual coils may be seen (Fig. 14A); the spines are up to 4 mm. long. In intertexta there are so many-celled glandular hairs on the pod surface (Fig. 16); the pods are somewhat larger, 12-15 mm. in diameter; the spines are bending bow-shapedly at 90° to the side surface of the pod so that the boundaries of individual coils are covered and not discernable (Fig. 14C); the spines are somewhat longer, up to 6 mm. Similarities of the two taxa consist of their general appearance as to stems, leaves, flowers, and preference for heavy moist soils as the growing sites. Lesins and Lesins (1963) reported the similarity of pollen grain morphology in these taxa.

MATERIALS AND METHODS

The ciliaris maternal was represented by two accession, Nos. 57 and 1032. The former had been obtained from Israel, the second collected on the Island of Sardinia. They both conformed with the characterization as given above. The intertexta with the red leaf marking was Acc. No. 239 mentioned already in the chapter on inheritance of leaf marking in intertexta.

Considering the findings on dominance of leaf marking in intertexta the crosses again were carried out without a complete emasculation of the

Fig. 14-17 Fig. 14 - pod of M. *ciliaris* (A), of M. *intertexta* (C) and of their hybrid (B). Fig. 15 - section of a pod of M. *ciliaris* showing the pod hairs. Fig. 16 - section of a pod of M. *intertexta* with no hairs. Fig. 17 - section of a pod from the F_1 hybrid M. *ciliaris* x M. *intertexta* with hairs.



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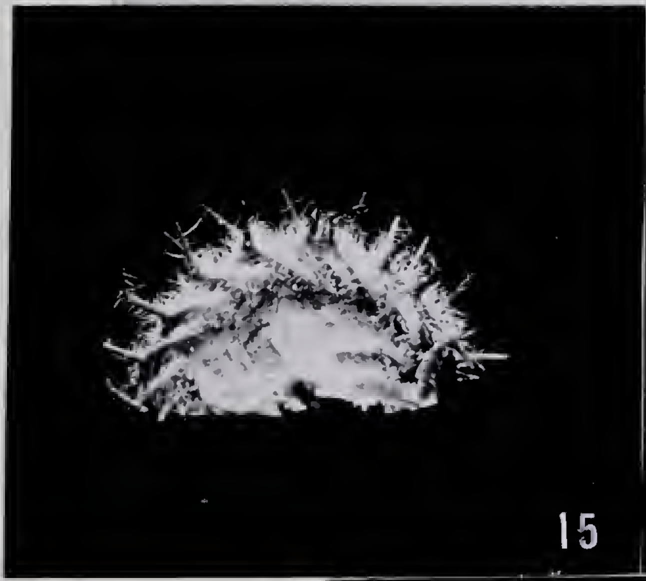

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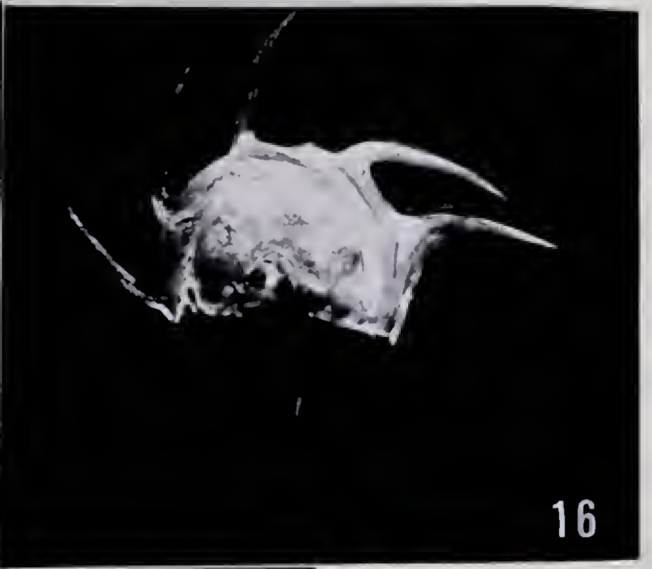

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ciliaris flowers used as the maternal plants. The red marking found in some progeny plants indicated that they were hybrids.

The characters of red marking on leaves and of many-celled hairs on the pods were used for inheritance studies in an attempt to assess the course of inheritance and the normality of chromosome segregation. The rather disturbing factor in this investigation was the extreme susceptibility of intertexta as well as of ciliaris to the alfalfa mosaic virus. The disease was fairly widely spread in the greenhouse before it was diagnosed by the legume virus specialist at Can. Dept. Agr. Vancouver, Dr. Pratt (personal letters). The affected plants, depending on the stage of infection, got stunted, stem tips dried up, often without flowering and seed setting, and eventually died. The disease was especially detrimental to the study of the character of glandular hairs which could be determined only on plants reaching past-flowering stage. It was also felt that the leaf marking may be made unrecognizable in plants from early-infested seedlings. Due to the effect of disease the pollen viability was rather variable. It was found that in the greenhouse under conditions favoring infestation some intertexta 239 plants showed only about 25% good pollen whereas plants of the same strain in the field where plants were health showed 75% good pollen. The transmitting agents of the virus in the greenhouse were mainly pea aphids which could not be fully controlled even with regular fumigation and insecticide sprays.

RESULTS

Segregation data on leaf marking character in F_2 and F_3 generations are given in Table VI.

As may be seen in Table VI the F_2 generations from the three

TABLE VI

Segregation of leaf marking character in F_2 and F_3 generations in *M. ciliaris* x *M. intertexta* crosses

Item No.	F_2 Plants				Number of F_3 Plants		
	Origin and designation	With marking	Without marking	P Value (3:1)	With marking	Without marking	P Value 3:1 for segregating families
	ciliaris 57 x *intertexta* 239						
1	1	1			44	15	0.90-0.95
2	2	1			43	14	0.90-0.95
3	3		1		0	37	
4	4	1			44	13	0.70-0.80
5	5	1			39	12	0.80-0.90
6	6		1		0	47	
7	7	1			38	22	$P < 0.05$
8	8	1			51	0	
9	9		1		0	40	
	Total	6	3				
	ciliaris 1032 x *intertexta* 239						
10	1	1			17	5	0.80-0.90
11	2	1			-	-	
12	3	1			22	14	0.05-0.10
13	5	1			45	0	
14	6		1		0	16	
15	7	1			42	16	0.50-0.70
16	8	1			15	7	0.30-0.50
17	9	1			-	-	
18	10	1			26	0	
19	11	1			11	0	
20	12	1			9 (11)	14 (12)	
21	13	1			19	0	
22	14	1			-	-	
	Total	12	1				
	F_1 (*ciliaris* 57 x *intertexta* 239) x F_1 (*ciliaris* 1032 x *intertexta* 1032)						
23	1	1			33	0	
24	2	1			15	8	0.20-0.30
25	3	1			9	0	
26	4	1			16	2	0.10-0.20
27	5		1		0	28	
	Total	4	1				
	Grand Total	22	5	0.50-0.70			

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different sources fit the ratio of 3 marked to 1 without marking taken individually as well as together. The recessive character, without marking, bred true as all the 5 F_3 families from F_2 plants without marking did not segregate. A further evidence for the simple inheritance due to one pair of alleles "M" and "m" may be seen in the distribution of F_3 families. A theoretical ratio of 5.5 non-segregating marked : 11 segregating marked: 5.5 non-segregating not marked would be expected for F_3 families and, 5, 12 and 5 plants were found for the respective classes, this fitting the theoretical ratio with P = 0.90 - 0.95. The two families, items Nos. 19 and 25, had to be excluded because of small family size. Taken separately the segregating F_3 families were in agreement with the expected 3:1 ratio except two (items 7 and 20 in Table VI). It may be suspected that the severe virus infection precluded the expression of the leaf marking in some heterozygotes. Not a single plant from these families reached the flowering stage. That there were plants genetically belonging to the marked group could be seen when one of the families (item 20) was transferred from the greenhouse, where only 9 plants were found marked, to a strongly lit growth chamber and, an additional 2 plants could be identified as marked.

The character of many-celled glandular hairs on the pods was found in F_1 generations though neither the glandular hairs nor the spines were exactly as strongly expressed (Figs. 17, 14B) as in the respective parents. The character of hairiness could not be as completely followed up as that for leaf marking because pod hairs could be observed only at a later stage of plant development. At that time a number of plants had died or did not come to flowering due to the virus disease. The data for segregation in F_2 and F_3 families are given in Table VII.

As may be seen from Table VII the F_2 generations from the three different origins separately and together indicate that one gene governs the character. The dominant allele H ("H" for hairs) determining the presence of hairs, and the "h" their absence. Some variation in F_2 plants was noted: some plants having thicker, some thinner coverage of hairs. The distribution of F_3 families, theoretically expected as 4.75 with hairs not segregating: 9.5 with hairs segregating: 4.75 without hairs not segregating, was found to be 6:9:4 respectively; not counting the small-sized families where appearance of without-hairs-individuals at 3:1 ratio could not be established with significant reliability. Eight of the segregating F_3 families showed a good fit with the expected 3:1 ratio, one (item 26), however, did not. This family derived from an F_2 plant, classified as hairless and consequently considered as homozygous recessive, produced two hairy plants in a progeny of 17. There is no ready explanation for the phenomenon. It may be speculated that the intercross of the F_1 from two different <u>ciliaris</u> strains in that particular F_2 plant had a genic combination that suppressed the genetically-present hairiness of that plant. It may be visualized as the two <u>ciliaris</u> strains having two separate closely linked genes for hairiness H_1 and H_2. Then there may be a combination in F_2 $H_1h_2h_2h_2$ where one dominant factor is covered up by three recessives. In its progeny 1/4 of plants may have two dominant alleles and hence show the hairs. The hypothesis however, would need a larger material and further generations to be proven.

The phenotypic classification of 27 F_2 plants for the characters leaf marking and hairiness jointly resulted in 18 plants with marked leaves and hairy pod-spines, 4 plants with marked leaves and not hairy pod-spines, 4 plants with not-marked leaves and hairy pod-spines, and 1 plant with not

TABLE VII

Segregation data on many-celled glandular hair character in F_2 and F_3 generations from the cross *ciliaris* x *intertexta*

	F_2 Plants				Number of F_3 Plants		
Item No.	Origin and designation	With hairs	Without hairs	P Value (3:1)	With hairs	Without hairs	P Value for segregating (3:1)
	ciliaris 57 x *intertexta* 239						
1	1	1			53	0	
2	2	1			36	15	0.30-0.50
3	3	1			31	0	
4	4	1			40	16	0.50-0.70
5	5	1			40	11	0.50-0.70
6	6	1			30	14	0.20-0.30
7	7		1		0	55	
8	8		1		0	49	
9	9		1		0	38	
	Total	6	3				
	ciliaris 1032 x *intertexta* 239						
10	1		1		0	17	
11	2	1			-	-	
12	3	1			-	-	
13	5	1			6	1	0.50-0.70
14	6	1			10	3	0.80-0.90
15	7	1			23	0	
16	8	1			21	0	
17	9	1			1	?	
18	10	1			15	0	
19	11	1			7	3	0.20-0.30
20	12	1			-	-	
21	13	1			9	?	
22	14	1			-	-	
	Total	10	1				
	F_1 (*ciliaris* 57 x *intertexta* 239) x F_1 (*ciliaris* 1032 x *intertexta* 239)						
23	1	1			22	3	0.10-0.20
24	2	1			18	5	0.70-0.80
25	3	1			9	0	
26	4		1		2	15	
27	5	1			24	0	
	Total	4	1				
	Grand Total	22	5	0.50-0.70			

marked leaves and not hairy pod-spines. Both characters being monogenically controlled, the assumption of independence of transmission results in 15.1: 5.1:5.1:1.7 respectively. The agreement with assumption that the factors responsible for appearance of the two characters are located in two separate chromosomes is good (P = 0.70 - 0.80).

CONCLUSIONS

The inheritance of the two characters indicated that the chromosomes in the two taxa were homologous to the degree that the transmission and segregation of the respective two major genes, M-m and H-h, followed the course expected for the crossing partners belonging to the same species. A few disagreements with the expected may have been caused by the virus disease in leaf marking inheritance, and in different genetic set-up for hairiness within the M. ciliaris material.

Crosses in M. rigidula All.

The taxon M. rigidula comprises a number of morphological variations which sometimes have been considered as separate species. The basis for separation has been differences in spininess, in hairiness of pods and in the size and shape of pods. Lesins and Lesins (1963) reported differences in pollen shape in two strains of rigidula. Since pollen shape generally is considered a rather stable character its inheritance was of special interest. In addition, spininess and red marking on leaves were the characters which served as indicators whether the chromosome pairing and distribution were following the pattern generally considered as indicating same-species relationship.

MATERIALS AND METHODS

M. rigidula strain Acc. No. 489 originated from Iraq, it had a red patch at the base of leaflets (Fig. 20) and as expected transmitted the character as a dominant to its offspring. This strain when used as male parent readily marked its true descendants, so that the crossing could be performed without the injurious emasculation. The crossing was done as indicated in the chapter dealing with M. striata x M. littoralis crosses. The pollen grains of this strain were triangular (Fig. 25). The pods were medium sized, 6-7 mm. diameter, with spines (Figs. 27A, 28C). Three other strains were used as female parents. The strain Acc. No. 1324 had been collected in Lebanon. It had no red leaf marking and was without spines (Figs. 27D, 28A). Strain, Acc. No. 1661, collected on the Island of Corsica had no leaf marking (Fig. 18), with spines (Fig. 27C) similar to No. 489, its pollen grains were cylinder-shaped (Fig. 24). An additional strain Acc. No. 993 from the Island of Capri had not the red marking on the leaves, but the pods were larger, 7-8 mm. in diameter, and with longer spines (Fig. 27B). All the strains were diploids with 2n=14 chromosomes.

The meiotic studies were carried out using the staining procedure given by Lesins (1954) and Lesins and Lesins (1963).

In M. rigidula as in ciliaris x intertexta, the disease was destructive. Tips of branches developed small leaves, internodes became shorter, the branch began to dry up and eventually the plant died. Segregation ratios may have been influenced by the disease.

Figs. 18-23 Leaf marking in M. rigidula. Fig. 18 - Acc. No. 1661. Fig. 20 - Acc. No. 489, Fig. 19 - F_1 from them. Figs. 21-23, F_2 of 1661 x 489: well-marked leaflets (Fig. 21), with incomplete marking in middle leaflet only (Fig. 22), with traces of marking at only the bases of leaflets (Fig. 23).


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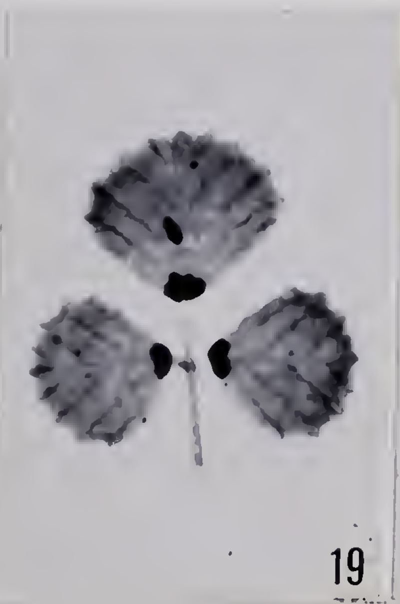
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Figs. 24-26 Pollen grains of M. *rigidula*: No. 1661 (Fig. 24), No. 489 (Fig. 25), F_1 hybrid (Fig. 26).


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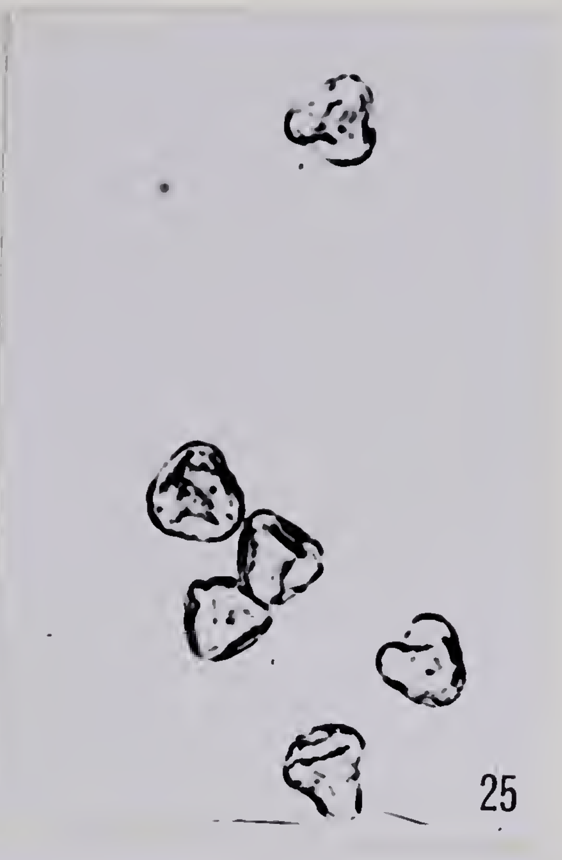
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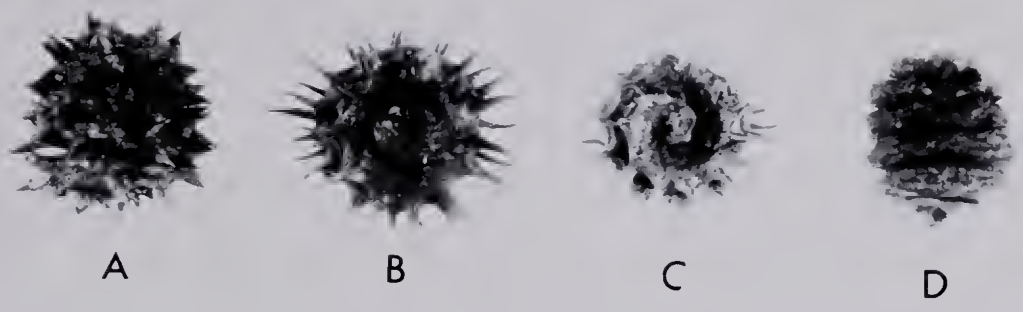
Fig. 27 Pods of M. *rigidula* 489 (A), M. *rigidula* 993 (B), M. *rigidula* 1661 (C), and M. *rigidula* 1324 (D).

Fig. 28 Pods of M. *rigidula* 1324 (A), F_1 of *rigidula* 1324 x *rigidula* 489 (B), and *rigidula* 489 (C).



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RESULTS

Inheritance of red leaf marking

Some of the F_1 plants with leaf marking derived from No. 489 ♂ x other three strains (Fig. 19) were used to produce the F_2 generation. The results on leaf marking inheritance are shown in Table VIII.

TABLE VIII

Segregation of red leaf marking in F_2 generation in crosses between M. rigidula Nos. 993, 1324, 1661 (no marking) x No. 489 (marking)

Item No.	Origin and No. of F_1 Plants	Number of F_2 Plants		P Value (3:1)
		With marking	Without marking	
	M. rigidula 993 x M. rigidula 489			
1	1	10	4	
2	2	16	1	
	Total	26	5	0.20-0.30
	M. rigidula 1324 x M. rigidula 489			
3	11	12	1	
4	12	10	2	
5	15	13	1	
6	16	14	2	
7	17	15	2	
8	22	10	2	
9	32	12	2	
10	33	18	2	
11	34	8	2	
12	35	18	7	
13	40	15	3	
14	42	8	2	
15	44	11	7	
	Total	164	35	P=0.01-0.02
	M. rigidula 1661 x M. rigidula 489			
16	1	23	4	
17	4	84	20	
18	14	32	14	
19	31	5	1	
	Total	144	39	0.20-0.30

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As may be seen in Table VIII, there is a shortage of plants without marking in all three different crosses taken separately as well as together. The shortage is significant in the cross 1324 x 489. This is a rather interesting case as all the 13 items taken separately do not deviate from 3:1 ratio significantly though the number of segregates is small. However, 11 of them show some shortage of non-marked plants. There does not seem to be any other explanation but that the true 3:1 ratio has been distorted by some disturbing factors. For one thing there is the high mortality of M. rigidula material as indicated in Table IX.

TABLE IX

Survival of seeds from F_1 plants from different inter-crosses of M. rigidula strains.

Origin of F_1	Seeds from F_1				
	No. put for germination	Germinated		Survived to grown plants	
		Number	%	Number	% of put to germination
No. 993 x 489	58	54	93	31	57
No. 1324 x 489	380	323	85	199	62
No. 1661 x 489	311	285	92	189	66

Another possibility of error is the misclassification of the material. There was no difficulty in identification of F_1 hybrids as to the presence of leaf marking, whereas in one set of F_2 plants which were grown in the growth cabinet, 6 individuals could be classified as not being marked and 26 as being marked, but 6 could not be classified (Fig. 23). A third possibility may be the different viability of gametes, though in this cross the pollen viability was not investigated.

Inheritance of spininess

The M. rigidula No. 1324 had no spines (Fig. 28A). In crosses with No. 489 the red leaf marking again identified the F_1 hybrids in an early stage of development, the characteristic spininess of M. rigidula 489 showed up in F_1 hybrids (Fig. 28B) later at full development of the plants. The segregation in F_2 for spininess is given in Table X.

TABLE X

Segregation of factor for spininess in M. rigidula 1324 x M. rigidula 489

Item No.	Designation of F_1 Plants	Number of F_2 Plants		P Value 3:1
		With spines	Without spines	
1	11	8	4	
2	12	9	3	
3	15	10	4	
4	16	11	4	
5	17	13	3	
6	22	9	3	
7	32	9	3	
8	33	13	6	
9	34	8	2	
10	35	15	5	
11	40	14	4	
12	42	7	1	
13	44	8	5	
	Total	134	47	0.70-0.80

As may be seen in Table X there was a good fit for the ratio 3:1. This would indicate that the two M. rigidula strains are so closely related that their chromosome pairing and distribution of alleles are as normal as expected in crosses involving the same species. This supports also the assumption made previously considering the leaf marking inheritance, namely that the marking actually was 3:1 ratio disturbed by some inviability linked with the marking character.

Inheritance of pollen shape and of viable pollen

The pollen shape was cylindrical in M. rigidula 1661 (Fig. 24) and appeared triangular (pyramid-shaped) in No. 489 (Fig. 25). Generally at determination of pollen shape there may be two factors involved. One factor is the maternal environment which is diploid in nature, where the pollen grains develop; the other is the genetic constitution of the pollen grain itself which is haploid in nature. Thus assuming that the triangular shape would be determined by a gene "A", the non-triangular (cylindrical) by "a", then in the hybrid the maternal tissue would be Aa and if the A is dominant and the pollen shape determined by the maternal tissue then all pollen should be triangular though half of them will carry no allele A. On the other hand, if the pollen would be determined by the genetic constitution of the pollen grain itself then half of the pollen grains, those with allele A should be triangular, the other half with the allele "a" should be cylindrical. There are reports that both possibilities may occur (Bateson 1919, Mangelsdorf 1931).

In the present case, as may be seen in Table XI, in F_1 hybrids there were found triangular as well as cylindrical pollen grains (Fig. 26). Moreover, the proportion of differently shaped grains in different F_1 plants were variable ranging for triangular pollen from 23 to 66% of all classified grains. Some variations may be attributable to the sampling error though 50 to 100 grains were tested in each case. Nevertheless it was obvious that there was no dominance of one of the parental shapes.

It could then be assumed that the genetic constitution of pollen itself may have determined the pollen shape. The average proportion of pollen from all F_1 plants was half (49%) triangular (Table XI, the other half (51%) being non-triangular (cylindrical). The great variation 23-66%,

TABLE XI

Distribution of triangular* pollen in parents and in crosses

Plant material	Number of plants tested	Class limits of % triangular pollen and number of plants in them																						Average % triangular pollen **
		0	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	71-75	76-80	81-85	86-90	91-95	96-99	100	
M. rigidula 1661	4	4																						0
M. rigidula 489	4																						4	100
F_1's _M. rigidula_ 1661 x _M. rigidula_ 489	21						3		1		4	1	1	7	3	1								49
F_2 plants from F_1 Pl. 1 (23% triang.)	27		1	1	3		2	1		2			1	1	1	2		1		3	3	3	2	56
F_2 plants from F_1 Pl. 4 (63% triang.)	102	5	6	7	4	5	2	5	1	2	1		1	2	2	4	9	5	10	3	8	15	5	59
F_2 plants from F_1 Pl. 14 (44% triang.)	45	1	3	2	1	4	3	1	3		3	2	1		2		2	2	1	7	2	5		53
F_2 plants from F_1 Pl. 31 (45% triang.)	6													1						1	1	3		88
Total for F_2 plants	180	6	10	10	8	9	7	7	4	4	4	2	3	4	5	6	11	8	11	14	14	26	7	58

* The complement with respect to 100 consists implicitly of cylindrical pollen.
** The average % were found directly from the non-classified data.

then might be ascribed to sampling error.

The ratio of homozygotes to heterozygotes in F_2 generation of a cross segregating at n independent loci ($n = 1, 2, ...$) is $1 : (2^n - 1)$. With $n = 3$ and $n = 4$ the expected ratios are 1:7 and 1:15 respectively. Assuming that the pollen shape is controlled by four independent loci out of 180 plants (see Table XI) 11.25 would be non segregants and 168.75 segregants. The observed numbers are 6 + 7 = 13 and 167 respectively. If it is considered that the non segregants are of two types, one producing only cylindric pollen and the other only triangular pollen and if it is assumed that they are in equal proportions we may write:

	Cylindric	Mixed	Triangular	Total
Observed	6	167	7	180
Expected	5.625	168.750	5.625	180

$P = 0.80\text{-}0.90$

The basic number of individuals in F_2 population of a cross, involving 4 loci is 256.

Although based on 180 observations only, there is a tendency of genotypes producing a certain percent of pollen of one type to be as frequent as the genotypes producing the same percent of pollen grains of second type, resulting roughly in 50 triangular, 50 cylindric pollen grains ($P > 0.10$) in our case.

Another rather interesting feature in this cross was the poor pollen viability (Table XII). Parental plants had the following amounts of viable pollen: No. 1661 had 76 to 82%, and No. 489 had 96 to 99%. The F_1 hybrids had only 14% viable pollen on the average. The fluctuations being from 9 to 21%. It should be indicated that the viability in pollen shape reported above was unlikely due to differences in pollen viability

because the 4 F_1 plants varied very little, 14 to 19% pollen viability (Table XII). The chromosomal translocations were suspected when the poor F_1 pollen was detected. To check this possibility mother cells were studied. The results are given in Table XIII.

As may be seen in Table XIII that the chromosome configurations at metaphase I, which stage is considered the most informative, was normal : 94% were bivalents. At anaphase I the distribution to the poles was 98% without laggards. This means that the reason for abortive pollen grains may be due to small deficiencies and duplications in homologous chromosomes. The same effect may be expected from lethal genes rendering a gamete inviable if two lethals initially in separate chromosomes happen to come together in one chromosome following crossing over. The few other than bivalents formation in MI were four plates having 5 II + 1 IV, two plates with 6 II + 2 I and one plate with 5 II + 1 III + 1 I. Some of the configurations are shown in Figs. 29-33.

The normal inheritance of the factor for leaf-marking Table VIII has obviously not been closely linked with any of the factors causing pollen abortion. It appears also that the factors for pollen shape have not been closely linked with the pollen viability as the one-shape pollen and high pollen viability, characteristic of parents, has not been found together in F_2 plants as seen in Table XIV.

Generally the pollen quality has been improving rather rapidly from 14% viable pollen on the average in F_1 to 52% on the average in different F_2 plants as may be seen in Table XII.

TABLE XII

Percent of viable pollen in parents and in crosses

Plant material	Number of plants tested	Class limits of % viable pollen and number of plants in them															Average % viable pollen*
		0	1-5	6-10	11-15	16-20	21-25	26-35	36-45	46-55	56-65	66-75	76-80	81-85	86-95	96-99.5	
M. rigidula 1661	4												2	2			79
M. rigidula 489																4	98
F_1's *M. rigidula* 1661 x *M. rigidula* 489	43			4	27	10	2										14
F_2 from F_1 No. 1 (16.3% viable pollen)	27							2	5	7	6	6	1				55
F_2 from F_1 No. 4 (14.0% viable pollen)	105	3			2	6	6	16	17	23	18	6		3	5		45
F_2 from F_1 No. 14 (19% viable pollen)	45							4	14	12	7	4	1	1	1	1	51
F_2 from F_1 No. 31 (14.5% viable pollen)	6								2		2	1	1				59
Total for F_2 plants	183	3	0	0	2	6	6	22	38	42	33	17	3	4	6	1	52

* The average % were found directly from the non-classified data.

TABLE XIII

Chromosome configurations at meiotic stages in F_1 plants of *M. rigidula* 1661 x *M. rigidula* 489

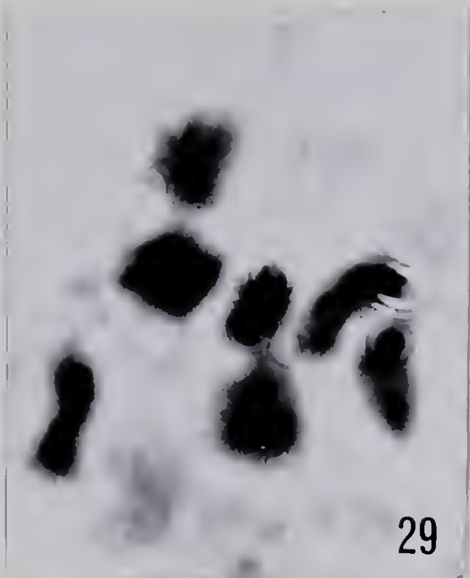
F_1 Plant No.	Metaphase I		Anaphase I		Metaphase II		Anaphase II	
	7 II	Other	Without laggards	With laggards	7+7+7+7	Other	Without laggards	With laggards
1	2		16				6	
2	22	2	22					
3	13	1					7	
6	32	4	51	1	2			
11	12		14	1				
14			11					
24	11							
26	16							
29	1		6					
Total	109	7	120	2	2		13	
% Normal	94		98					

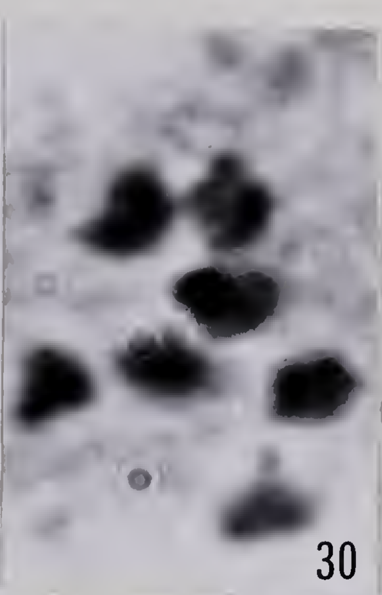
TABLE XIV

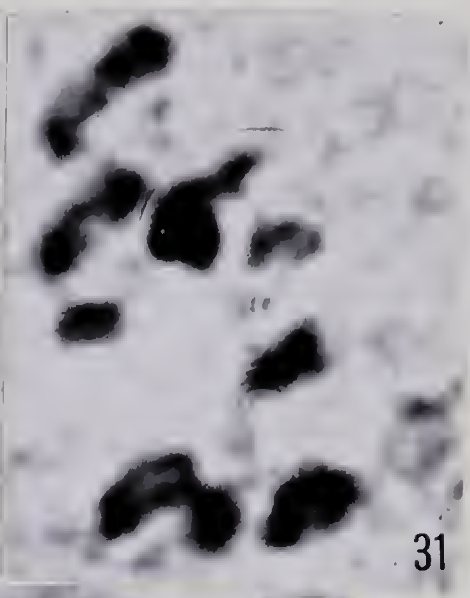
Pollen viability in F_2 plants with one-shape pollen grains

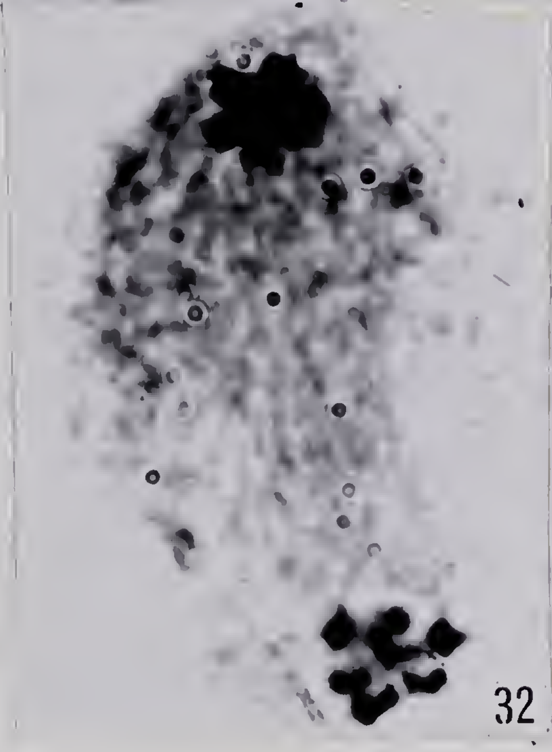
Designation of F_2 plant	Pollen shape	% viable pollen	Designation of F_2 plant	Pollen shape	% viable pollen
6	cylindrical	67	48	triangular	48
22	"	59	61	"	66
33	"	43	72	"	47
34	"	44	76	"	58
37	"	56	78	"	32
41	"	44	4	"	58
			17	"	70

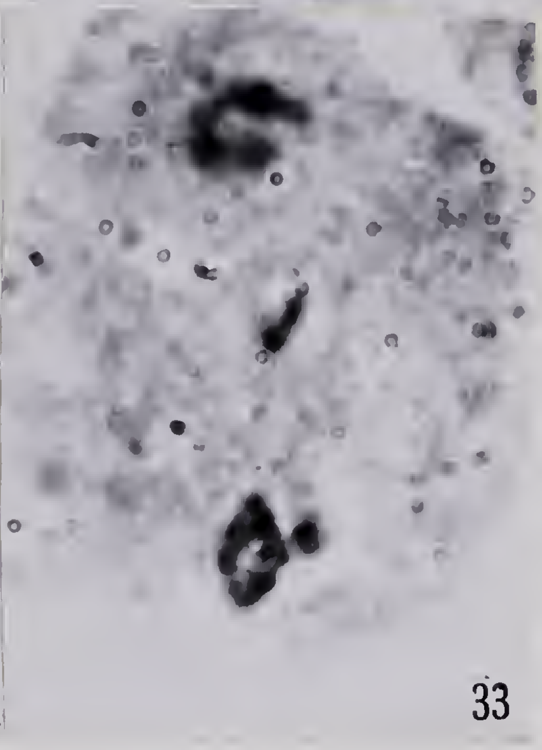
Figs. 29-33 Chromosome configurations at meiotic stages, Figs. 29, 30 - M I with 7 bivalents, Fig. 31, M I with 5 II and 4 I, Fig. 32 - A I with normal distribution to poles, Fig. 33 - A I with one laggard in the middle.


29


30


31


32


33

CONCLUSIONS

In the three different crosses involving on one side *M. rigidula* No. 489 ♂ and Nos. 993, 1324, 1661 on the other side, there has been a good agreement with the expected ratios for normal chromosome pairing and segregation for at least in one character: leaf marking or spininess. This would indicate that the tested strains may be considered belonging to the same species. In cases where the agreement with the expected and observed ratios has not been good i.e. for leaf marking in No. 1324 x 489, it may be assumed that different viability of zygotes may have caused the distorted ratios.

Regarding the poor pollen viability in F_1 in the cross of strains No. 489 ♂ and No. 1661, it may be assumed that spatially widely separated strains, in this case from Iraq and from Corsica, have accumulated small chromosomal rearrangements which at crossing-over in F_1 plants give a large portion of abortive pollen. The differences in chromosomal constitution have likely been incorporated in the two different strains by chance not as a barrier against intercrossing. This may be deduced from the observation that the viability of pollen was rapidly recovering in F_2 when the proportion of the heterozygotes was halfed with respect to F_1.

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